

Attorney's Docket No. 3477-116

10-29-03

PATENT

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In The United States Patent And Trademark Office

In re Pugh et al.
Appl. No.: 09/029,872
Filed: June 29, 1998
For: ARTIFICIAL STABILIZED COMPOSITION OF CALCIUM
PHOSPHATE PHASES PARTICULARLY ADAPTED FOR
SUPPORTING BONE CELL ACTIVITY

Confirmation No.: 6664
Group Art Unit: 3738
Examiner: P. Prebilic

October 28, 2003

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APPEAL BRIEF TRANSMITTAL
(PATENT APPLICATION - 37 C.F.R. § 1.192)

- Transmitted herewith, in **triplicate**, is the APPEAL BRIEF in this application, with respect to the Notice of Appeal filed on August 28, 2003.
- ☒ Applicant claims small entity status.
- Pursuant to 37 C.F.R. § 1.17(c), the fee for filing the Appeal Brief is:
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Respectfully submitted,

Melissa B. Pendleton

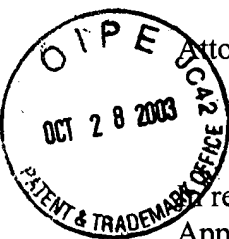
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APPEAL BRIEF UNDER 37 CFR § 1.192

This Appeal Brief is filed pursuant to the "Notice of Appeal to the Board of Patent Appeals and Interferences" filed August 28, 2003.

1. ***Real Party in Interest.***

The real party in interest in this appeal is Millenium Biologix, Inc., the assignee of the above-referenced patent application.

2. ***Related Appeals and Interferences.***

There are no related appeals and/or interferences involving this application or its subject matter.

3. ***Status of Claims.***

The present appeal involves Claims 1, 2, 6, 10, 12, 13, 22, 23, 25-29, 32-35, 37 and 38. Claims 3-5, 7-9, 11, 14-21, 24, 30, 31, 36, and 39-46 have been canceled. The claims at issue, namely, Claims 1, 2, 6, 10, 12, 13, 22, 23, 25-29, 32-35, 37 and 38, are set forth in the attached appendix.

4. ***Status of Amendments.***

An Official Action was mailed March 3, 2003, finally rejecting pending Claims 1, 2, 6, 10, 12, 13, 22, 23, 25-29, 32-35, 37 and 38.

Applicants are filing an Amendment After Final concurrently with this Appeal Brief to correct an apparent typographical error in the dependency of Claims 6 and 10. In particular, Claims 6 and 10 are amended to depend from process Claim 13, and not composition Claim 1.

Applicants respectfully request entry of this amendment. As noted in the Amendment After Final, the amendments do not raise new issues for consideration by the Examiner because the subject matter of the amended claims has been before the Examiner throughout prosecution. In addition, the amendment places the claims into better condition for consideration on appeal because the amendment corrects an apparent typographical error in the dependency of the claims.

Applicants also note that the final Official Action requested that the specification be amended to properly recite the claim of priority. Upon a favorable finding on appeal, Applicants intend to amend the specification to reflect the claiming of priority from U.S. Application No. 60/003157, filed Sept. 1, 1995; U.S. Application No. 08/576,238, filed on December 21, 1995; and PCT/CA96/00585, filed Aug. 30, 1996.

5. ***Summary of the Invention.***

The present invention is directed to a bioactive artificial sintered composition (claims 1, 2, 6, 10, 12, 23, 25, 26, 32, and 38) and processes of making the composition (claims 13 and 22). The invention also includes an implantable calcified bone matrix that includes the composition of the invention (Claims 27-29); implantable devices coated with the composition of the invention (Claim 33); implantable devices consisting essentially of the composition of the invention (Claim 34); and methods of using the composition to culture functional bone cells (Claim 35) and ex vivo engineering an implant (Claim 37).

The composition of the invention is in the form of a powder or bulk material. The material includes stabilized insoluble tricalcium phosphate. The stabilized insoluble tricalcium phosphate results from stabilizing entities present uniformly throughout the entire composition. The stabilizing entities include silicon, among other entities. The inventors have surprisingly

discovered that the claimed stabilized insoluble tricalcium phosphate phases are capable of supporting bone cell activity thereon.

It is the stabilized, insoluble nature of the tricalcium phosphate that is formed which is of significance. Page 13, lines 20-21 of the present application. Applicants were the first to discover that the presence of stabilizing entities can stabilize the composition and prevent its degradation in physiological fluids. See Claim 1, reciting a stabilized insoluble tricalcium phosphate and Claim 12, reciting a composition insoluble in physiological fluids of pH of approximately 6.4 to 7.3. Surprisingly the composition is stable in the presence of various aqueous media even though alpha tricalcium phosphate is supposed to be soluble in water. Page 5, lines 15-17 and 19-20 of the application. See also page 9, line 28- page 10, line 4. Although not wishing to be bound by any explanation of the invention, it is currently believed that the formation of insoluble calcium silicate entities at the surface of each particle limits the reversibility of the reaction and plays a role in preventing the solubility of alpha tricalcium phosphate in aqueous physiological media. Page 15, lines 20-23.

That Applicants' material is insoluble is surprising in view of the conventional view that alpha tricalcium phosphate is soluble, and thus not desired for therapeutic applications. The record before the Examiner, including Applicants' specification and art cited by the Examiner, demonstrates that one skilled in the art would consider alpha tricalcium phosphate to be a soluble product. For example, the present application states that "[i]n the development of calcium phosphate based coatings, α -TCP has not been a great subject of attention because of its degradation in physiological fluids due to its relatively high solubility." Page 14, lines 1-3. See also page 5, lines 15-17, which note that the product is stable in the presence of various aqueous media, even though alpha tricalcium phosphate is supposed to be soluble in water. See also pages 77 and 79 of the Ruys article, cited by the Examiner.

Not only is the tricalcium phosphate material insoluble in physiological fluids. The insoluble tricalcium phosphate is also bioactive, i.e., resorbable by osteoclasts and promotes secretion of mineralized bone matrix by osteoblasts. Applicants have found that the claimed compositions of the present application support osteoblastic bone growth over and throughout structures made of the composition. The materials also promote natural controlled extracellular

resorption of the composition by osteoclasts, while avoiding non-specific chemical and/or cellular dissolution and/or degradation, in a process resembling that of normal bone turnover. See pages 10, 19 and 22-23 of the present application. Applicants were the first to develop and characterize a stabilized composition that behaves similar to natural bone, and which in fact integrates with the natural bone over time so that an implant formed of the composition is progressively replaced by natural bone.

The stabilized composition can be used to provide a range of coatings, powders and bulk ceramic pieces that share a common surface globular microporosity and an internal microporosity. In addition, the bulk ceramics also can have a macroporosity within the structure in order to provide an artificial three-dimensional bone tissue similar to that found *in vivo*. The composition, made in any form, encourages the activity of bone cells cultured thereon and also allows for the development of *ex vivo* engineered artificial bone tissues to use as bone grafts.

Bone is a complex mineralizing system composed of an inorganic or mineral phase, an organic matrix phase, and water. Calcification of bone depends on the close association between the organic and inorganic phases to produce a mineralized tissue, and disturbance of the natural balance of bone formation and resorption leads to various bone disorders. Because the invented composition encourages the activity of bone cells, the invention has applications in medical diagnostics for the assessment of normal and abnormal bone cell activity as well as for medical therapeutics including bone and dental tissue replacement and repair as well as for *ex vivo* bone graft tissue engineering.

Applicants have discovered that the presence of uniformly distributed stabilizing entities throughout a hydroxyapatite substance stabilizes tricalcium phosphate compounds within the substance and prevents the degradation of the tricalcium phosphates in physiological fluids. The unique stabilized and insoluble tricalcium phosphates are recited in each of the pending composition and method claims. The claimed compositions and methods stand in contrast to tricalcium phosphate containing compositions of the past, in which the tricalcium phosphate content, particularly the α -TCP content, was soluble and biodegradable.

The stabilized insoluble artificial bioactive composition is the first such composition which supports both osteoclast and osteoblast activity and which allows for the reliable

assessment of the physiological activities of both cell types as well as for the development of both diagnostic and therapeutic strategies.

6. ***Issues.***

The issues in the present appeal are as follows:

(1) Whether Claims 1, 2, 6, 12, 13, 22, 23, 25, 32, 34 and 38 are properly rejected under 35 U.S.C. § 102 as being anticipated by Ruys (article entitled "Silicon-Doped Hydroxyapatite"), when Ruys does not teach each and every one of the claimed elements;

(2) Whether Claims 10, 26 and 33 are properly rejected under 35 U.S.C. § 103(a) as being unpatentable over Ruys, when there is no basis or motivation to make the proposed modification; and

(3) Whether Claims 27-29, 35 and 37 are properly rejected under 35 U.S.C. § 103(a) as being unpatentable over Ruys in view of Davies (WO 94/26872), when there is no basis or motivation to make the proposed combination.

7. ***Grouping of Claims.***

Claims 1, 2, 6, 10, 12 and 38 are directed to a bioactive artificial sintered composition ("the composition") comprising a powder or bulk material of stabilized insoluble tricalcium phosphate phases. Claims 23, 25, 26 and 32 are directed to an embodiment of the invention in which the composition may be provided as a microporous polycrystalline structure. Claim 34 is directed to an embodiment of the invention in which the composition forms an implantable device. Claims 1, 2, 6, 10, 12, 23, 25, 26, 32, 34, and 38 should be considered together.

Claim 33 is directed to an implantable device coated with the composition. Claim 33 stands separately because this claim defines subject matter that renders it patentable independently of Claims 1, 2, 6, 10, 12, 23, 25, 26, 32, 34, and 38.

Claims 13 and 22 are directed to a process for stabilizing an artificial sintered composition of calcium phosphate phases. Claims 13 and 22 stand separately because these claims define subject matter that render them patentable independently of Claims 1, 2, 6, 10, 12, 23, 25, 26, 32, 34, and 38; and Claim 33.

Claims 27-29 are directed to an implantable calcified bone matrix comprising a structure formed from the composition, and a calcified bone matrix secreted by osteoblasts on said structure. Claims 27-29 stand separately because these claims define subject matter that render them patentable independently of Claims 1, 2, 6, 10, 12, 23, 25, 26, 32, 34, and 38; Claim 33; and Claims 13 and 22.

Claim 35 is directed to a method for the culturing of functional bone cells, the method comprising applying a suspension of bone cells in physiological media to the composition, provided as a substrate. Claim 35 stand separately because this claim defines subject matter that renders it patentable independently of Claims 1, 2, 6, 10, 12, 23, 25, 26, 32, 34, and 38; Claim 33; Claims 13 and 22; and Claims 27-29.

Claim 37 is directed to a method for the *ex vivo* tissue engineering of a mineralized collagenous implant. Claim 37 stands separately because this claim defines subject matter that renders it patentable independently of Claims 1, 2, 6, 10, 12, 23, 25, 26, 32, 34, and 38; Claim 33; Claims 13 and 22; Claims 27-29; and Claim 35.

8. ***Argument.***

(1) Claims 1, 2, 6, 12, 13, 22, 23, 25, 32, 34, and 38 are not properly rejected under 35 U.S.C. 102 because Ruys does not teach each and every element of the claimed invention.

Claims 1, 2, 6, 12, 13, 22, 23, 25, 32, 34 and 38 stand rejected under 35 U.S.C. § 102 as being anticipated by Ruys. The Examiner argues that Ruys produces the same material as claimed, and thus it would inherently have the same resorbability and in vivo response as claimed. Applicants submit, however, that the Ruys material is not the same as the claimed composition and thus does not inherently have the same properties.

The present invention includes a bioactive artificial sintered composition for supporting bone cell activity. As recited in Claim 1, the composition is in the form a powder or bulk material. The powder or bulk material includes stabilized insoluble tricalcium phosphate, resulting from stabilizing entities present uniformly in the material. The stabilizing entities include silicon, among other entities.

It is the stabilized, insoluble nature of the tricalcium phosphate that is formed which is of significance. Page 13, lines 20-21 of the present application. Applicants were the first to discover that the presence of stabilizing entities can stabilize the composition and prevent its degradation in physiological fluids. See Claim 1, reciting a stabilized insoluble tricalcium phosphate and Claim 12, reciting a composition insoluble in physiological fluids of pH of approximately 6.4 to 7.3. Surprisingly the composition is stable in the presence of various aqueous media even though alpha tricalcium phosphate is supposed to be soluble in water. Page 5, lines 15-17 and 19-20 of the application. See also page 9, line 28- page 10, line 4. Although not wishing to be bound by any explanation of the invention, it is currently believed that the formation of insoluble calcium silicate entities at the surface of each particle limits the reversibility of the reaction and plays a role in preventing the solubility of alpha tricalcium phosphate in aqueous physiological media. Page 15, lines 20-23.

Not only is the tricalcium phosphate material insoluble in physiological fluids. The insoluble tricalcium phosphate is also bioactive, i.e., resorbable by osteoclasts and promotes secretion of mineralized bone matrix by osteoblasts. Applicants have found that the claimed compositions of the present application support osteoblastic bone growth over and throughout structures made of the composition. The materials also promote natural controlled extracellular resorption of the composition by osteoclasts, while avoiding non-specific chemical and/or cellular dissolution and/or degradation, in a process resembling that of normal bone turnover. See pages 10, 19 and 22-23 of the present application. Applicants were the first to develop and characterize a stabilized composition that behaves similar to natural bone, and which in fact integrates with the natural bone over time so that an implant formed of the composition is progressively replaced by natural bone.

That Applicants' material is insoluble is surprising in view of the conventional view that alpha tricalcium phosphate is soluble, and thus not desired for therapeutic applications. The record before the Examiner, including Applicants' specification and the cited Ruys article, demonstrates that one skilled in the art would consider alpha tricalcium phosphate to be a soluble product. For example, the present application states that "[i]n the development of calcium phosphate based coatings, α -TCP has not been a great subject of attention because of its

degradation in physiological fluids due to its relatively high solubility.” Page 14, lines 1-3. See also page 5, lines 15-17, which note that the product is stable in the presence of various aqueous media, even though alpha tricalcium phosphate is supposed to be soluble in water. See also pages 77 and 79 of the Ruys article. Here Ruys teaches that TCP is an undesired product because of its biodegradability, citing S.R. Radin and P. Ducheyne, J. Mater. Sci. Mater. Med., 3 (1992) 33, a copy of which is attached.

Contrary to the position taken by the Examiner, the Ruys article does not describe the production of the same material as claimed. Rather, Ruys describes the production of conventional TCP, which is soluble, i.e., biodegradable, in physiological fluids. Ruys nowhere teaches or suggests the production of a stabilized insoluble tricalcium phosphate as claimed which exhibits bioactivity allowing resorption by osteoclasts and promoting secretion of mineralized bone matrix by osteoblasts.

Ruys describes its product as follows. On page 79, Ruys states its process results in isomorphous substitution of silicon in the HAp (hydroxyapatite). The resultant product is actually a mixture of four products: calcium silicophosphate; tricalcium phosphate $\text{Ca}_3(\text{PO}_4)_2$, including β (beta) and α (alpha) phases; silicon-doped HAp; and silicon glass. Page 79. Although the Ruys product can include tricalcium phosphate, the TCP product differs from the claimed composition. Ruys explicitly states that the TCP phase is undesirable as biodegradable (page 77 noted above) and thus should be kept to a minimum to eliminate the possibility of biodegradability in vivo (page 79, also noted above). Thus if anything, the Ruys article actually teaches away from the claimed invention, stating that TCP phases are to be avoided because of their undesired biodegradability properties.

TCP as produced by Ruys is not only biodegradable in physiological media. It also has not been demonstrated to have any “bioactivity” relating to osteoclast resorption or osteoblast matrix secretion. Ruys indicates on page 79 that the studies may allow “future assessment of the effects of silicon on the bioactivity of HAp through clinical trials”. Ruys does not even suggest assessing the bioactivity as herein described for an insolubilized tricalcium phosphate product.

In summary, the claimed invention provides stabilized insoluble tricalcium phosphate exhibiting bioactivity allowing resorption by osteoclasts and promoting secretion of mineralized

bone matrix by osteoblasts. In contrast, the Ruys product includes biodegradable tricalcium phosphate. As such, it cannot inherently exhibit the bioactive properties of the claimed composition, i.e., it cannot inherently exhibit the same resorbability and in vivo response as claimed. Indeed, Ruys teaches away from the claimed invention, stating that the production of tricalcium phosphate is undesirable because of its known biodegradability.

Ruys does not recite each and every element of Claim 1. Ruys accordingly cannot anticipate Claim 1. Inasmuch as the remaining claims subject to this rejection incorporate the composition of Claim 1, they also are not anticipated by Ruys. Accordingly, the Examiner has not established a sustainable anticipation rejection and the rejection of Applicants' claims should be reversed.

(2) Claims 10, 26 and 33 are not properly rejected under 35 USC Section 103(a) because there is no basis in the art for modifying Ruys.

Claims 10, 26 and 33 stand rejected under 35 USC Section 103(a) as obvious over Ruys alone. Claim 10 recites a specific stabilizing entity of the bioactive, stabilized insoluble tricalcium phosphate material of Claim 1. Claim 26 recites specific morphology and dimensions of the material of Claim 1, specifically, rounded granules with a lateral dimension of about 0.5 to 1 μm . Claim 33 recites an implantable device coated with the composition of Claim 1. Contrary to the position set forth by the Examiner, Ruys nowhere suggests modifying its product to result in these claimed aspects of the invention.

As discussed above, the product of Ruys is not the same as claimed. The Ruys product includes soluble tricalcium phosphate. In contrast, the claimed composition of Claim 1 is a bioactive stabilized insoluble tricalcium phosphate material. As such, the claimed composition allows resorption by osteoclasts and promotes secretion of mineralized bone matrix by osteoblasts. Applicants were the first to develop and characterize a stabilized composition that behaves similar to natural bone, and which in fact integrates with the natural bone over time.

The Examiner argues that it would have been obvious to substitute tetraethyl silicate of Ruys with tetrapropyl silicate of Claim 10. Yet, Ruys nowhere suggests substituting any reagent

set forth therein. That one could have selected a different reagent is not the standard for determining obviousness, absent some suggestion to make the proposed modification. Any motivation or suggestion to make this modification is missing. Accordingly, the Examiner's conclusion requires an improper hindsight analysis based on Applicants' own teachings.

The Examiner also argues that particle size of the resulting product of Claim 26 is an obvious design choice. Yet Ruys nowhere teaches or suggests any morphology or dimensions for the product described therein. Accordingly, to conclude that the morphology and dimensions recited in Claim 26 are obvious requires the skilled artisan to (1) select a specific morphology (rounded) when there is none indicated, and also (2) select a specific dimension (about 0.5 to 1 μm), again where none is given as a starting point. That one could have selected these variables again is not the standard for determining obviousness absent some suggestion. Any such requisite motivation is missing in this case, and accordingly the Examiner's conclusion in this regard also requires an improper hindsight analysis.

Even if one were to modify the silicon source, or select from nothing specific morphology and dimensions, the result would still not be the same as claimed. As discussed above, Ruys produces biodegradable tricalcium phosphate, which is known in the art to be soluble in physiological fluids and thus undesirable for therapeutic applications. In contrast, the claimed composition differs from the Ruys product and includes bioactive stabilized insoluble tricalcium phosphate.

Ruys also nowhere suggests any specific applications for the products produced therein. Ruys certainly does not suggest coating an implantable device with the product described therein, much less coating an implantable device with tricalcium phosphate. Indeed, if anything, Ruys teaches away from in vivo applications of tricalcium phosphate, describing such products as biodegradable and thus undesirable. Therefore, Ruys teaches away from the claimed invention of claim 33 and as a result cannot render this claim obvious.

Obviousness cannot be established by combining or modifying the teachings of the prior art to produce the claimed invention, absent some teaching, suggestion or incentive supporting the modification or combination. *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F2d 1572, 1577, 221 USPQ 929, 933 (Fed.Cir. 1984). A Section 103 rejection presumes the

existence of differences between the subject matter claimed and the teachings of the prior art. Thus, the Examiner must be able to point to something in the prior art that suggests in some way a modification of a particular reference or a combination with another reference to arrive at the claimed invention. Absent such a showing in the prior art, the Examiner has impermissibly used the Applicants' teaching to hunt through the prior art for the claimed elements and combine them as claimed. *In re Laskowski*, 871 F2d 115, 117, 19 USPQ 2d 1397, 1398 (Fed.Cir. 1989).

This is what the Examiner has done in the present case. Ruys does not suggest the specific silicon reagent of Claim 10; or composition morphology or dimensions of Claim 26. That one could have selected the recited reagent, morphology or dimensions does not render Claims 10 and 26 obvious because there is no suggestion to make this modification. The only suggestion to do so is in Applicants' own specification. Likewise Ruys does not suggest coating an implant with the product produced therein. Indeed, Ruys, if anything, teaches away from the use of its product in vivo because it includes biodegradable tricalcium phosphate, which is stated to be undesired. Accordingly, the Examiner has not established a sustainable prima facie obviousness rejection. For this reason, the rejection of Claims 10, 26 and 33 should also be reversed.

(3) Claims 27-29, 35 and 37 are not properly rejected under 35 USC Section 103(a) because there is no basis in the art for combining Ruys with Davies.

Claims 27-29, 35 and 37 are rejected as obvious in view of the teachings of Ruys in view of Davies (WO 94/26872). Claims 27-29 recite an implantable calcified bone matrix that includes the composition of Claim 1 forming a structure for supporting the same. Claim 35 recites a method for culturing functioning bone cells using the composition of Claim 1 as a substrate. Claim 37 recites a method for ex vivo engineering of a mineralized collagenous implant using the composition of Claim 1.

The Examiner acknowledges that Ruys does not teach the presence of bone cells or their excreted materials. Not only does Ruys not teach or suggest this aspect of the claimed invention. As discussed above, the product of Ruys is not the same as claimed. The Ruys product includes

soluble tricalcium phosphate. In contrast, the claimed composition of Claim 1 is a bioactive stabilized insoluble tricalcium phosphate material. As such, the claimed composition allows resorption by osteoclasts and promotes secretion of mineralized bone matrix by osteoblasts. Applicants were the first to develop and characterize a stabilized composition that behaves similar to natural bone, and which in fact integrates with the natural bone over time so that an implant formed of the composition is progressively replaced by natural bone, thereby allowing a method of ex vivo engineering as claimed.

In contrast, the Ruys product includes biodegradable tricalcium phosphate. As such, the biodegradable tricalcium phosphate cannot exhibit the bioactive properties of the claimed composition. Indeed Ruys teaches away from the claimed invention, stating that the production of tricalcium phosphate is undesirable because of its known biodegradability. Because the tricalcium phosphate of Ruys differs from the claimed composition, it cannot inherently exhibit the same resorbability and in vivo response as claimed.

In addition, Ruys does not assess the bioactivity of the material produced in any manner. Thus Ruys cannot suggest the use of its material as an implant as recited in Claims 27-29, much less as a material suitable for a method of ex vivo engineering of an implant as recited in Claim 37. Ruys also does not recognize or suggest any benefit of its material in the culturing of functional bone cells as recited in Claim 35.

Davies cannot overcome the deficiencies of Ruys. Davies is directed to a different process than Ruys and further addresses a different problem than that set forth by Ruys. Thus there is no motivation to combine the teachings of the references as suggested by the Examiner.

Davies is directed to a calcium phosphate based thin film on a substrate for culturing bone cells thereon. The thin film is intended as an analytical device only for in vitro use, and not for in vivo applications.

The Examiner relies upon Davies to argue that it was known to prepare implants by culturing bone cells on the material or implant to test it. Davies, however, certainly did not contemplate that the thin film could be used as a component of an implantable calcified bone matrix or providing the same as a bulk material to ex vivo engineer an implant. Use of a thin

film as an analytical component, for example in an analytical kit (see abstract) is altogether different from using a material to make an implant for in vivo applications.

In addition, the thin film produced in accordance to Davies also differs compositionally from the claimed composition. The thin film can be prepared by applying a sol gel to a substrate, such as a quartz substrate and heating the coated substrate. In the example using quartz, this substrate was selected simply as a support for the thin film for analytical purposes and for its thermally tolerant properties. The Davies thin film is made up of a complex mixture of stratified calcium phosphate phases. This is discussed in the present application on page 17 with reference to Figures 6(a)-(c). Figure 6(a) shows the Davies thin film in cross section with an EDX analysis performed at various locations throughout the thin film. The regions shown are the interface of the film and the substrate, the intermediate region above the interface, and the top of the film. As seen in this analysis, the Davies thin film has varying compositions throughout and thus is a complex mixture of several different calcium phosphate phases. The amount of silicon present in the various regions also differs significantly. See Figures (c)(i) – (iii), which illustrate the different amounts of silicon distributed throughout the Davies product. Thus Davies illustrates a complex mixture of various calcium phosphate phases, in which silicon is not uniformly distributed as claimed. Rather, silicon is present in stratified form in varying concentrations in different regions of the film. In contrast, the claimed invention is directed to a tricalcium phosphate composition in which stabilizing entities, such as silicon, are uniformly distributed.

In summary, Ruys and Davies are directed to different processes for making different products and address different problems. There is no motivation to combine the teachings of these references. Indeed, Ruys teaches away from the claimed invention, stating that tricalcium phosphate is an undesired product because of its biodegradability. Accordingly, the Examiner has not established a sustainable prima facie obviousness rejection. For this reason, the rejection of Claims 27-29, 35, and 37 should also be reversed.

CONCLUSION

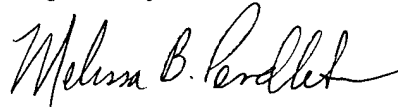
Ruys cannot anticipate the claimed invention because Ruys is directed to a product that differs from the claimed invention. The Ruys product includes biodegradable tricalcium

phosphate, stated to be undesired. In contrast, the claimed invention surprisingly provides bioactive stabilized insoluble tricalcium phosphate. Ruys does not assess what, if any, bioactivity the product produced therein has and certainly does not teach a product as claimed that is resorbable by osteoclasts and promotes secretion of mineralized bone matrix by osteoblasts. Accordingly Ruys does not teach every element of the claimed invention, and it cannot anticipate the claimed invention.

Ruys, alone or in combination with Davies, also does not render the claimed invention obvious. There is simply no motivation to make the modifications suggested by the Examiner, and in fact, Ruys teaches away from using tricalcium phosphate. Thus, the Examiner has failed to make a prima facie case of obviousness.

In view of the arguments presented above, it is accordingly respectfully submitted that the Board reverse the rejections of record and order the immediate allowance of all pending claims in this case.

Respectfully submitted,

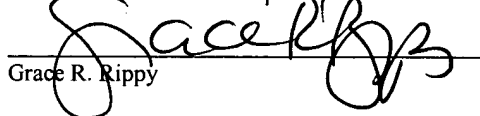


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Grace R. Rippy

APPENDIX

1. A bioactive artificial sintered composition for supporting bone cell activity, said composition comprising:

a powder or bulk material of stabilized insoluble tricalcium phosphate wherein the tricalcium phosphate is stabilized with stabilizing entities uniformly throughout the entire composition, and wherein said uniformly stabilized tricalcium phosphate is resorbable by osteoclasts and promotes secretion of mineralized bone matrix by osteoblasts,

wherein said stabilizing entities are selected from the group consisting of silicon entities, aluminum entities, barium entities, titanium entities, germanium entities, chromium entities, vanadium entities, niobium entities, boron entities and mixtures thereof.

2. A composition as claimed in claim 1, wherein said stabilized tricalcium phosphate is primarily alpha tricalcium phosphate.

3 – 5 - Cancelled

6. A composition as claimed in claim 1, wherein said stabilizing entities are provided as a solution.

7 – 9 - Cancelled

10. The composition as claimed in claim 6, wherein said stabilizing entities are a solution of tetrapropyl orthosilicate.

11 - Cancelled

12. A composition as claimed in claim 1, wherein said composition is insoluble in physiological fluids of pH of approximately 6.4 to 7.3.

13. A process for making the composition of claim 1, said process comprising:
doping and mixing a hydroxyapatite substance with a composition of stabilizing entities to uniformly distribute said stabilizing entities throughout said entire hydroxyapatite substance;

and sintering said uniformly doped hydroxyapatite substance;
wherein sintering converts at least a portion of said uniformly doped hydroxyapatite substance into primarily alpha tricalcium phosphate.

14 – 21 – Cancelled

22. The process of claim 13, wherein sintering is done at temperatures of about 900°C to 1100°C.

23. The composition of claim 1, where said composition is provided as a microporous polycrystalline structure.

24 – Cancelled

25. The composition of claim 23, wherein said structure has said globular morphology of Figure 14.

26. The composition of claim 25, wherein said morphology comprises rounded granules with a lateral dimension of about 0.5 to 1 μm .

27. An implantable calcified bone matrix comprising:
a) the composition of claim 1 forming a structure for supporting said bone matrix;
and

b) a calcified bone matrix secreted by osteoblasts on said structure.

28. An implantable calcified bone matrix of claim 27, wherein said matrix is free of bone cells including osteoblasts.

29. An implantable calcified bone matrix of claim 27, wherein said matrix includes a patients bone cells including osteoblasts.

30 – 31 – Cancelled

32. The composition of claim 23, wherein said composition has an internal macroporosity.

33. An implantable device coated with the composition of claim 1.

34. An implantable device consisting essentially of the composition of claim 1.

35. A method for the culturing of functional bone cells, said method comprising: applying a suspension of bone cells in physiological media to the composition of claim 1 provided as a substrate.

36 – Cancelled

37. A method of the *ex vivo* engineering of a mineralized collagenous implant, the method comprising the steps of:

- a) providing the composition of claim 1 as a bulk material;
- b) applying a suspension of osteoblasts on said composition and incubating for a time sufficient for said osteoblasts to secrete mineralized collagenous bone matrix on said bulk material; and
- c) implanting the product of step (b) in a patient.

38. The composition of claim 1, wherein said stabilizing entities are silicon.

39 – 46 – Cancelled.

Plasma spraying induced changes of calcium phosphate ceramic characteristics and the effect on *in vitro* stability

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Plasma spraying is a commonly used technique to apply thin calcium phosphate ceramic coatings. Special consideration is given to retaining the original structure of CPC particles. However, changes are possible. Thus this study focused on plasma spraying induced changes in material characteristics of commercial coatings and their influence on *in vitro* dissolution. All analysed coatings were found to undergo significant plasma spraying induced changes in phase composition, crystal structure, and specific surface area. The phase transformations depended on the starting particle characteristics. Specifically, β -TCP transformed to α -TCP. HA was dehydroxylated and transformed to oxyhydroxyapatite (OHA), and partly decomposed to α -TCP and tetra calcium phosphate. These transformations lead to a considerable increase of *in vitro* dissolution rates at physiological pH.

1. Introduction

Calcium phosphate ceramic (CPC) coatings on metallic implants have a threefold possible beneficial effect: enhancement of bone formation rates [1], the ability of bonding to bone [2], and the reduction of metal corrosion product release [3]. Plasma spraying, electrophoretic deposition and sputter coating are various methods to achieve CPC deposition [4].

Plasma spraying takes CPC particles through high temperatures. Special consideration has therefore been given to retaining the original crystalline structure of the CPC particles. It has been indicated that plasma spraying of hydroxyapatite leads to retaining at least 95% of the coating in this crystalline structure. This conclusion was arrived at by using X-ray diffraction (XRD) [5, 6]. However, in some of our previous work, more significant plasma sprayed induced changes of the crystal structure, and a concomitant change of specific surface areas were documented [7]. Thus in this study we focus on plasma sprayed induced changes in material characteristics of a number of plasma sprayed CPC coatings obtained from commercial sources, and the influence of these changes on the *in vitro* stability of these coatings.

2. Methods and materials

Two types of starting CPC powders were used for the study: hydroxyapatite (HA) and β -tricalcium phosphate (β -TCP). Both the starting powders and the plasma sprayed coatings were obtained from commercial sources. The powder and coating nomenclature are listed in Table I. The starting HAM and β -TCPPM had a mesh size of - 325, the starting HAF had a particle size 0.8 mm. The powders were sprayed

coated substrates. The average thickness of the CPC coatings was 70-80 μ m. The porous metallic coatings were obtained with either - 60 + 80 mesh spherical commercial purity (c.p.) Ti powders (ASTM F-67) or orderly oriented wire mesh (OOWM) of mesh size 16 and with a 0.50 mm diameter wire [8].

The XRD patterns were obtained on a Rigaku diffractometer (D/Max II, Danvers, MA) with CuK_α radiation at 45 kV, 35 mA, a 2 θ scanning rate of 1 deg min^{-1} . The instrument was equipped with a computerized diffractogram analyser. The infrared spectra were recorded on a Fourier transform infrared spectroscope (FTIR) Nicolet SDXC (Madison, WI). The powders or scraped plasma sprayed coatings were analysed as 1% powder-KBr mixtures in the diffuse reflectance operational mode.

The morphology of the CPC plasma sprayed coatings either onto flat or porous surface was determined by using scanning electron microscopy (Phillips, SEM 500, Eindhoven, The Netherlands). Scanning Auger electron spectroscopy (SAEMS, PHI Microprobe 600, Perkin-Elmer, Eden-Prairie, MN) was used to analyse the plasma sprayed coating surface composition.

The assessment of stability of either the starting CPC or the plasma sprayed coatings was performed in simulated physiological, calcium and phosphate free solution (a 0.05 M tris (hydroxy) methylamine-methane-HCl buffer) at pH 7.3, 37°C and 1 mg/1 ml mass to solution ratio, as described previously [9]. In brief, 10 mg of the powder, or metallic specimen; with 10 mg of coating were immersed into 10 ml of the solution for periods of time ranging from 15 min to 24 h. For each period of time, four separate specimens were used. The vials were placed onto a shake-table.

The amount of released calcium was measured in solution spectroscopy

TABLE I Characteristics of the CPC before and after plasma spraying

Nomenclature	Crystal structure	Used method of analysis	Specific surface area ($\text{m}^2 \text{g}^{-1}$)
HAM st. ^a	stoichiometric HA	XRD FTIR	0.64
HAM PS ^a	OHA + α -TCP + tetra CP	XRD	0.29
β -TCPM st. ^a	stoichiometric β -TCP	XRD	1.19
β -TCPM PS ^a	α -TCP + β -TCP remain	XRD	0.79
HAF st. ^b	stoichiometric HA	XRD FTIR	0.13
HAF PS ^b	OHA + traces α -TCP and tetra CP	XRD FTIR	0.02
β -TCPD st. ^c	the same as β -TCPM	XRD	1.19
β -TCPD PS ^c	β -TCP + α -TCP	XRD	-

Specimens kindly provided by ^aMittet, Warsaw, Indiana; ^bFeldmühle, Plochingen, Germany (Osprovit); ^cDe Puy, Warsaw, Indiana.

(AAS) (Perkin-Elmer, model 2380, Norwich, CT). The released phosphate was measured as the complex (molybdenum yellow) [10] in a UV-visible spectrophotometer (Biochrom, LKB 4053 Ultraspec, Cambridge, UK).

The specific surface areas were determined with the monolayer gas absorption technique (B.E.T.) by Micromeritics Co. (Norcross, GA) or Omicron Co. (Berkley Heights, NJ).

3. Results

3.1. Characteristics of the starting powders

Some characteristics of the starting CPC powders, including their crystal structures and specific surface areas are summarized in Table I. The starting HAM was stoichiometric, highly crystalline HA (as was confirmed by XRD and FTIR) with a low specific surface area (Table I). The FTIR spectrum of HAM (Fig. 1a) shows characteristic features of a completely crystallized HA, including well-pronounced OH⁻ peaks at 3572 and 633 cm⁻¹.

As follows from the FTIR analysis, the crystallization of the starting HAF was not complete (Fig. 2a). The spectrum shows a broad water band in the 3600–3000 cm⁻¹ range; the OH⁻ bands at 3572 and 633 cm⁻¹ are significantly smaller than those of the HAM spectrum. However, the specific surface area of HAF is very low. After calcination at 900 °C the FTIR spectrum of HAF is typical of stoichiometric, crystalline HA.

Starting β -TCPM powder was highly crystalline powder (as confirmed by XRD and FTIR) with a low specific surface area.

3.2. Characteristics of the plasma sprayed CPC

Some characteristics of the plasma sprayed coatings are also summarized in Table I. The FTIR spectra of HAM PS and HAF PS are shown in Figs 1b and 2b respectively. The XRD patterns of both plasma sprayed HAs and β -TCPs are represented in Fig. 3a to d.

Plasma spraying produced a dramatic change in the crystal structure of HAM as can be deduced from the

XRD and FTIR results. The starting HA was transformed into a mixture of apatite, tetracalcium phosphate (tetra CP) and α -TCP as follows from the XRD pattern (Fig. 3a). Not only is the X-ray diffraction analysis important, in addition, the infrared spectra must be reviewed critically, since they bring out features (Fig. 1b) which cannot be observed by XRD. The OH⁻ bands at 3572 and 633 cm⁻¹, characteristic of HA, have fully disappeared. The 961 cm⁻¹ band, characteristic of symmetric stretching of PO₄ group in HA, has disappeared, and two very weak bands at 966 and 946 cm⁻¹ have appeared. Two medium intensity bands at 603 and 568 cm⁻¹ and small shoulder can be seen in the bending vibration mode of the PO₄ group. All the above features can be assigned to oxyhydroxyapatite (OHA), of which the characteristic absorption bands were described before [11].

The FTIR spectrum of a single-phase OHA, which we produced by heating HA at 900 °C for 24 h in *vacuo*, is shown for comparative purposes in Fig. 4b. This spectrum shows the following characteristic features: a very weak OH⁻ band at 3672 cm⁻¹ and the absence of the hydroxyl band at 633 cm⁻¹; two medium intensity bands at 970 and 946 cm⁻¹; bands at 604, 532 and 568 cm⁻¹ and a shoulder at 556 cm⁻¹ in a bending vibration mode of the PO₄ group, and a medium intensity band at 481 cm⁻¹. Trombe and Montel [11] reported similar IR absorption bands for OHA. Specifically, ν_1 -bands at 965 and 935 cm⁻¹, ν_2 -bands at 600, 575, and 572 cm⁻¹, a shoulder at 560 cm⁻¹, and a 482 cm⁻¹ band in the low energy region. They found that the above features are more pronounced with a higher degree of dehydroxylation.

The full absence of the OH⁻ absorption bands on the FTIR spectrum of HAM-PS indicates a high degree of dehydroxylation of the OHA. The small additional features in the FTIR spectrum of HAM-PS (Fig. 1b), especially in a low energy region, could be associated with tetra CP and α -TCP. Thus the combined XRD and FTIR analysis identifies the HAM-PS structure as a mixture of OHA, tetra CP and α -TCP.

The XRD pattern of the HAF subsequent to plasma spraying indicates a highly crystalline apatitic structure, with some α -TCP and tetra CP as revealed by their barely perceptible characteristic peaks (Fig. 3b). Whereas the XRD pattern would lead one to believe

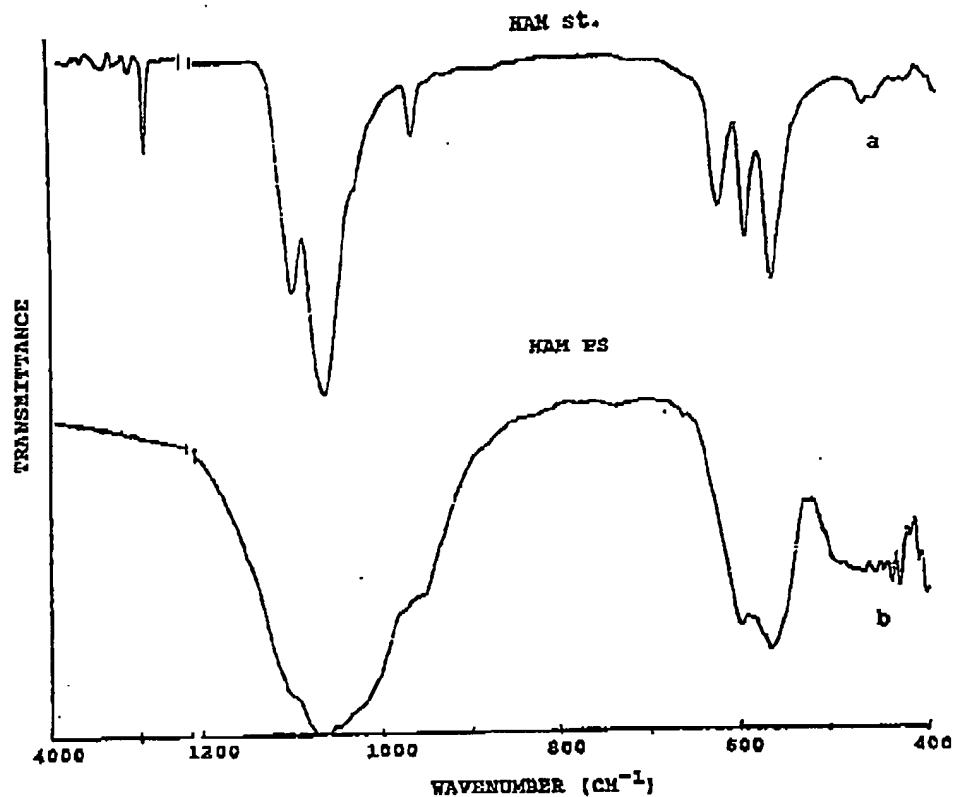
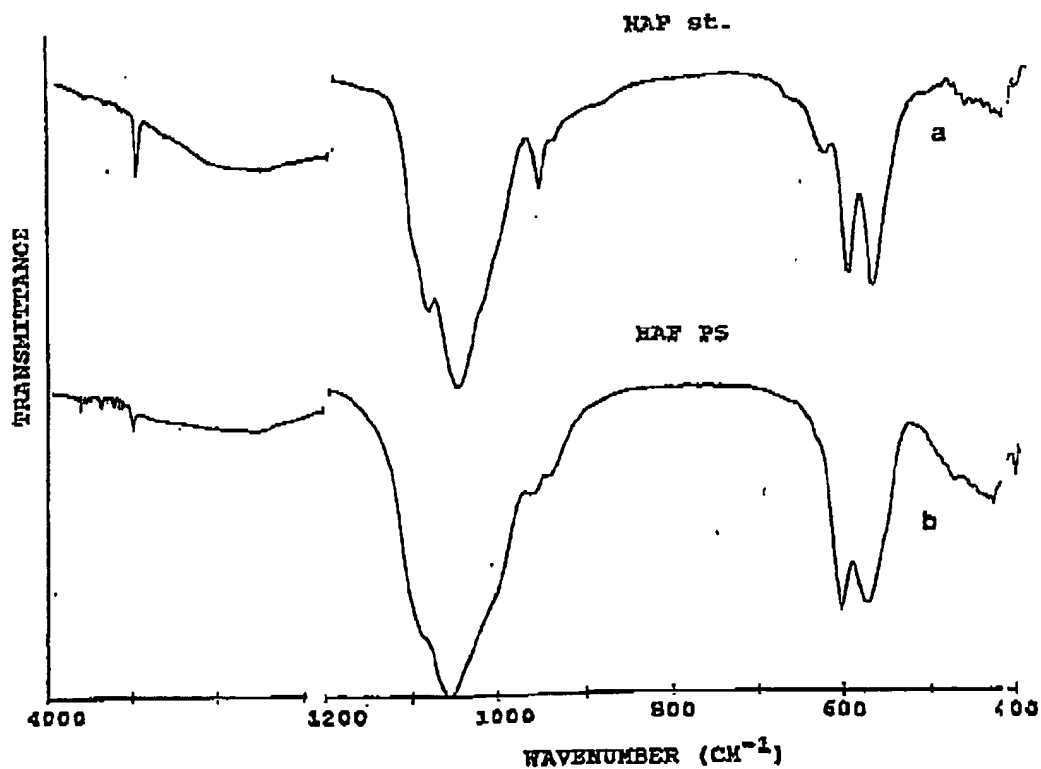


Figure 1 FTIR spectra of HAM starting (a) and HAM PS (b).



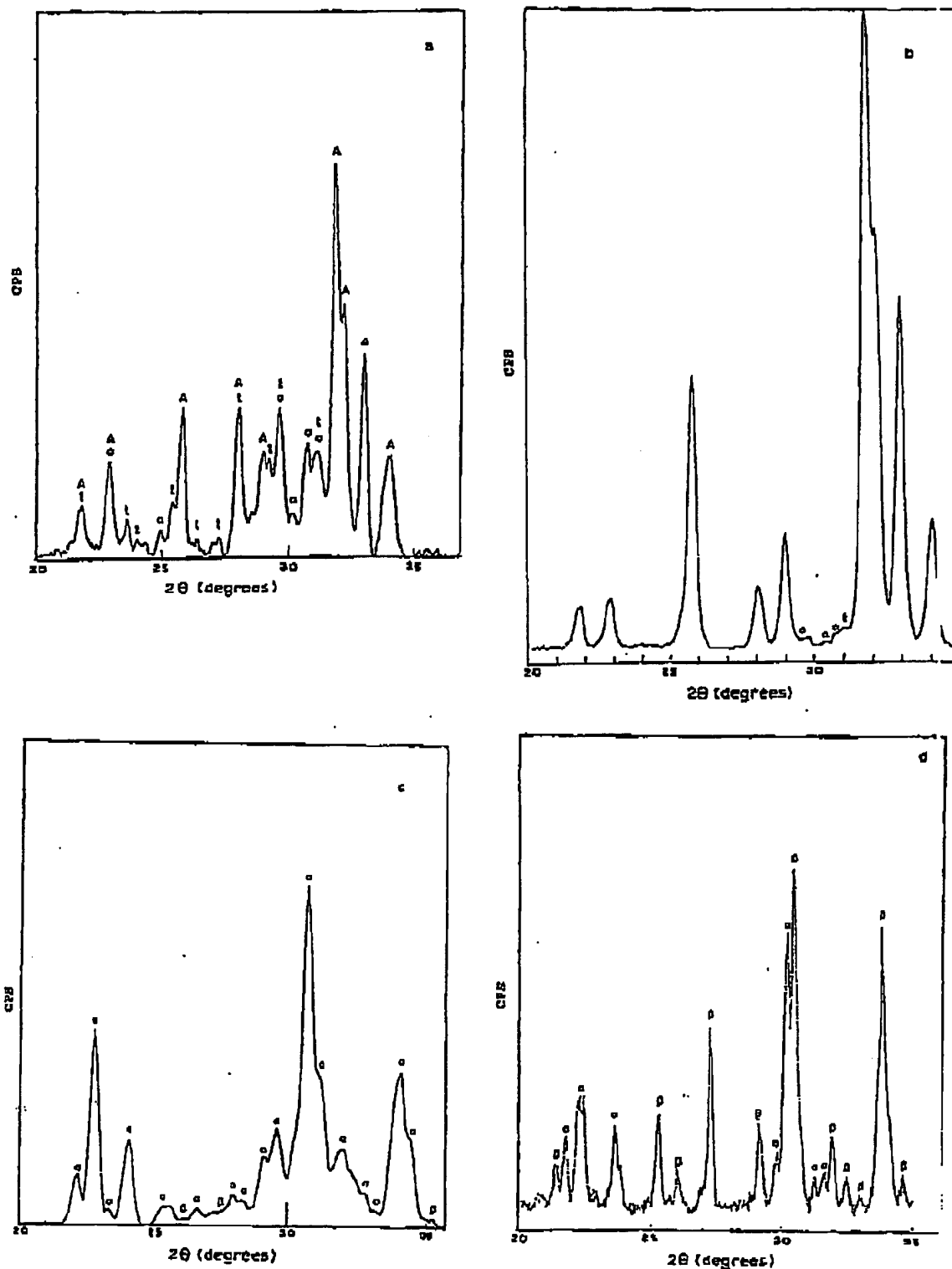


Figure 3 XRD spectra of plasma sprayed coatings: (a) HAM PS, (b) HAF PS, (c) β -TCPM PS, (d) β -TCPD PS.

that hydroxyapatite is largely retained, one must also consider the FTIR spectrum. After plasma spraying it differs significantly from that of the starting HAF, as can be seen by comparing Fig. 2a with Fig. 2b. The reduction of the 3572 cm^{-1} and the absence of the

bands at 962 and 948 cm^{-1} and the absence of the 961 cm^{-1} band are characteristic of the OHA structure. Thus the starting HAF underwent a partial dehydroxylation and transformation to OHA during the plasma spraying.

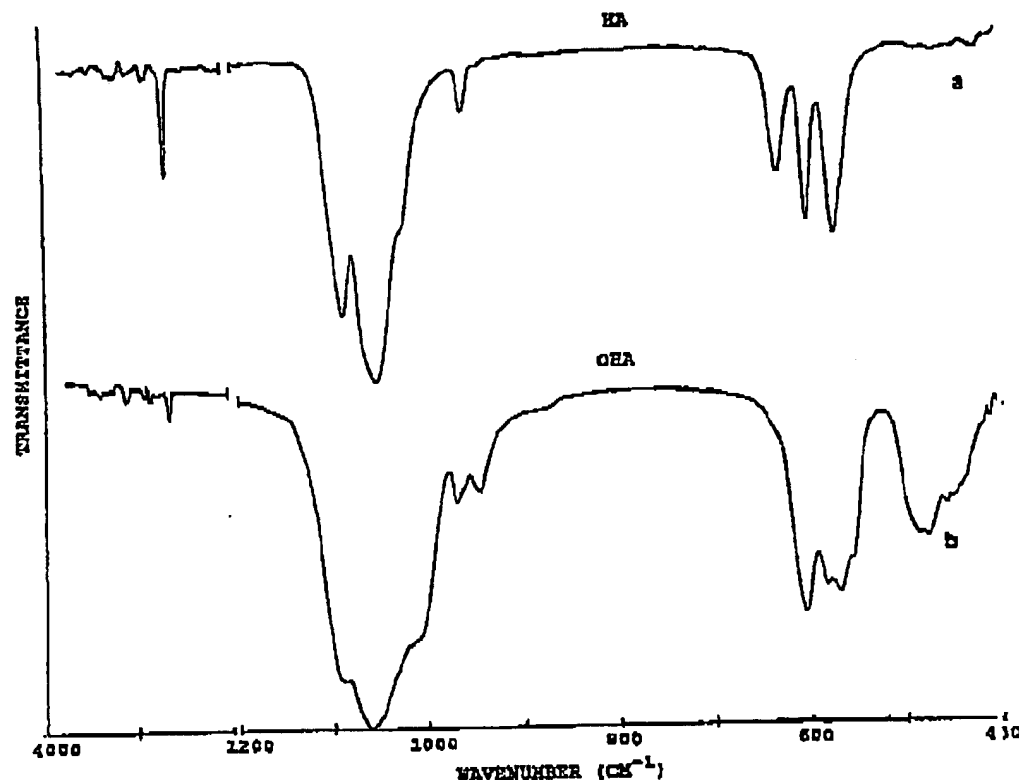


Figure 4 FTIR spectra of stoichiometric HA (a) and OHA (b), produced by heating the HA at 925 °C for 24 h in high vacuum in a Pt crucible.

to α -TCP, with some β -TCP retained, as indicated by XRD spectrum of Fig. 3c. Broadness of the peaks indicates that the degree of crystallinity of β -TCPM PS decreased with respect to the starting highly crystalline powder. The β -TCPD was transformed into a mixture of α -TCP and β -TCP as confirmed by XRD analysis (Fig. 3d).

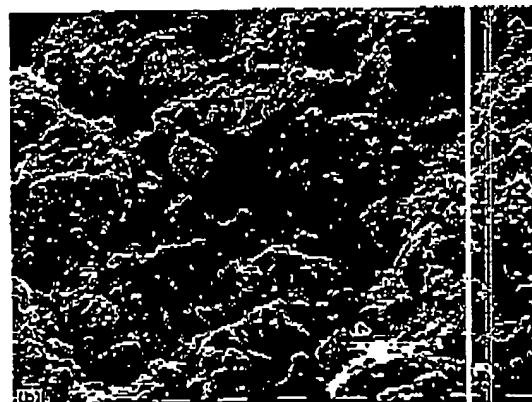
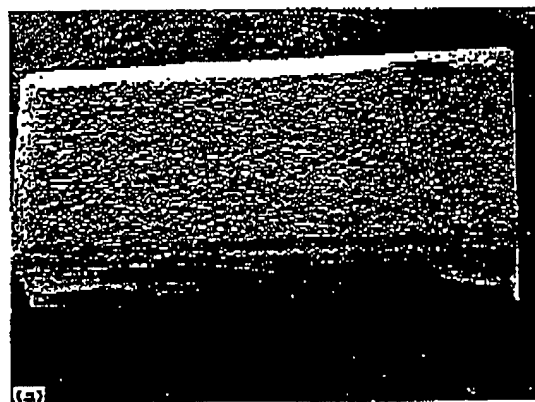
All the powders underwent reductions in specific surface areas, to a greater or lesser degree, due to plasma spraying (Table I).

3.3. SEM observations

The SEM micrographs of HAF PS coating onto a flat

metal surface are represented in Fig. 5a, b. The small magnification view (Fig. 5a) shows a uniformly covered metal substrate. However, at larger magnifications (Fig. 5b) the coating is non-uniform since it consists of large smooth areas with a glassy appearance in combination with irregular shape ceramic chips and randomly distributed pores of different size; multiple cracking can be observed as well.

SEM micrographs of the HAF plasma sprayed coating onto the OOWM porous surface are shown in Fig. 6a, b. At small magnifications (Fig. 6a) the coating seems uniform on both the substrate and OOWM. However, higher magnifications reveal that the areas of the substrate adjacent to the wires, i.e. the corners



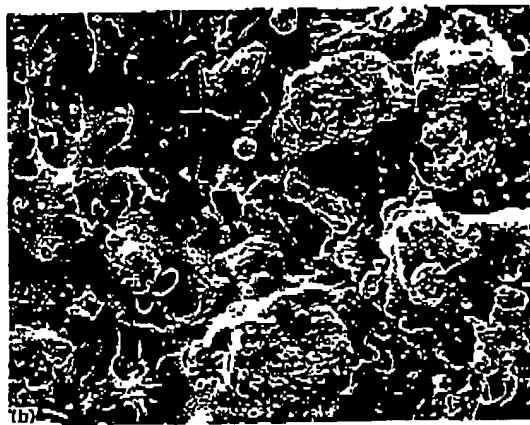
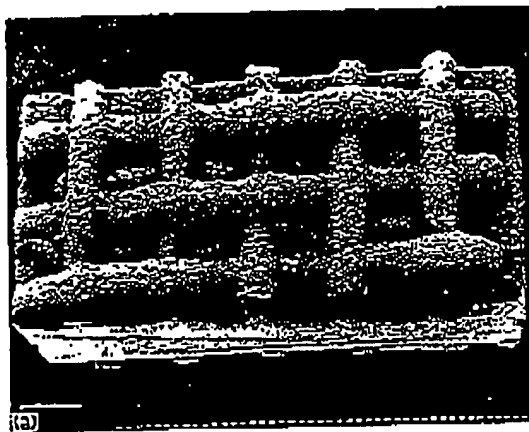


Figure 6 SEM view of the HAF-PS coating onto porous OOOVM coated surface at 10 \times (a), and details at 640 \times (b).

and the edges of the pores, were not coated, as well as the underside of the wires. The coating structure on the top of the wires (Fig. 6b) resembles that of the HAF-PS coating onto the flat surface. Specifically, one sees large particles with a glassy and highly deformed appearance, ceramic chips, random pores, and cracking.

The starting HAF particles were relatively large 0.8 μm diameter particles. The SEM appearances suggest that some of the large particles apparently underwent surface melting when being taken through the high temperatures in the carrier gas. Furthermore, these melted surfaces were quenched at the moment of impact with resulting cracking and breaking of the particles.

3.4. Coating surface analysis

The SAEMS HAF-PS coating surface survey revealed Ca, O and P peaks. A doublet low energy P peak was located at 92 and 109 eV. This observation corresponds to those reported for a phosphate group [12].

The Ca to P peak intensity ratio measured on the coating surface after 20 s of sputtering in the scan mode was found to be 6.0–6.2. The ratio is similar to that of a reference stoichiometric HA.

3.5. Dissolution behaviour of the CPC before and after PS

The concentrations of calcium and phosphate released from the CPC into the buffer solution for periods of time ranging from 15 min to 24 h are represented in Fig. 7a, b.

The starting HAM and HAF powders show the slowest dissolution rates as could be expected for stoichiometric well-crystallized HA with a low specific surface area. The starting β -TCPM shows a higher dissolution rate, since β -TCP is known to be more soluble than HA.

The dissolution rates of all plasma sprayed CPC, (either HAF-PS, HAM-PS or β -TCPM) were found to be significantly greater than those of the starting

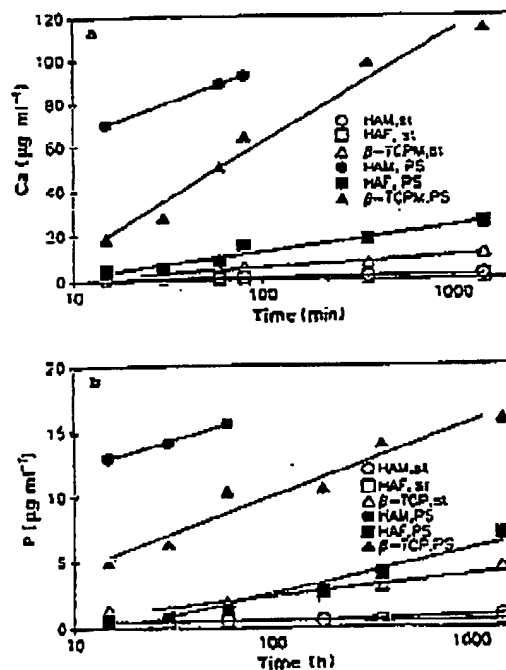


Figure 7 The Ca (a) and P (b) dissolution rates of the starting powders and plasma sprayed coatings in a 0.05 M Tris buffer solution at pH 7.3 and 37 °C.

released from the immersed HAF-PS is an order of magnitude greater than that from the starting HAF. The concentrations of calcium released from either HAM-PS or β -TCPM-PS appeared to be almost two orders of magnitude greater than those released from the starting powders. The HAM-PS and β -TCPM-PS were found to be the most soluble. The dramatic increase in dissolution rates of the PS CPC occurs in spite of the decrease in their specific surface areas (Table I).

To demonstrate whether the transformation of HA to OHA or β -TCP to α -TCP can alter the dissolution behaviour of the starting CPC, we performed an analogous immersion test of OHA and α -TCP. OHA- β -TCP was

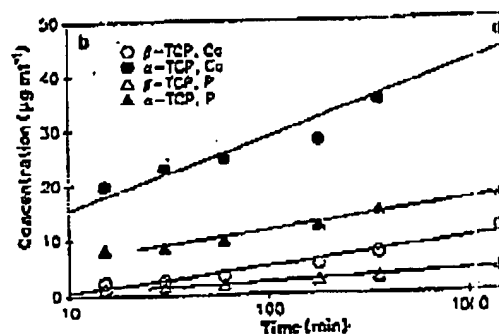
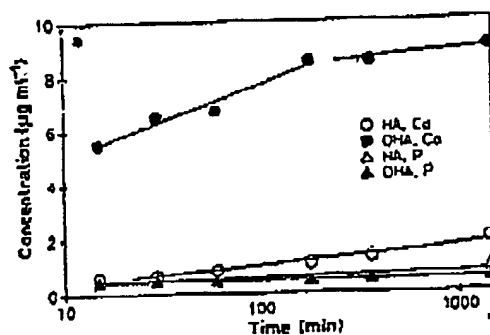


Figure 8 Changes in the dissolution rates of stoichiometric HA and β -TCP after full transformations to OHA (a) and α -TCP (b) respectively.

produced by heating β -TCPPM at 1420 °C for 6 h. The crystal structures of these two CPC were confirmed by XRD.

The dissolution rates of the HA and β -TCP powders before and after transformation to OHA and α -TCP are represented in Fig. 8a and b respectively.

A major increase in solubility of both HA or β -TCP subsequent to the transformation to OHA or α -TCP respectively, was found. Both the starting and the transformed powders were single-phase CPC with the same Ca/P ratio, yet their solubility depended significantly upon their crystal structures.

4. Discussion

4.1. Phase transformations

All the studied HA or β -TCP PS coatings on metal surfaces, obtained from commercial sources, underwent, to different extents, plasma spraying induced changes, including changes in crystal structures, specific surface area and morphology. Furthermore, these changes affected the stability, or the dissolution behaviour of the ceramic.

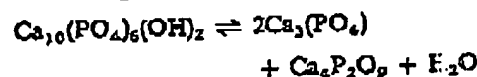
According to requirements suggested in the literature, satisfactory coatings must be dense, adherent, and with structures not altered irreversibly by the coating technique [13]; HA coatings must consist of a 95% highly crystalline HA [5, 6]. To achieve these requirements, major efforts have been concentrated upon retaining the original structure and composition of CPC particles during plasma spraying. Retaining at least 95% of the original CPC crystal structure seems possible according to some sources [5, 6]; however, irreversible plasma spraying induced changes in crystal structures of plasma sprayed CPC have been found as well. Specifically, β -TCP transformation to "amorphous alpha phase" [14] and HA decomposition with TCP phase formation [15] have been reported.

We observed significant differences among the HA or β -TCP PS coatings obtained from different sources, although all the starting particles were stoichiometric, single-phase compounds. Specifically, plasma spraying decomposed HAM into a mixture of apatite with a large amount of α -TCP and tetra CP (Fig. 3a). In contrast, HAF-PS consisted of 95% apatite with barely detectable extra phases (Fig. 3b). The β -TCPPM

TCPPD PS decomposed only partly and consisted of a mixture of β - and α -TCP (Fig. 1d).

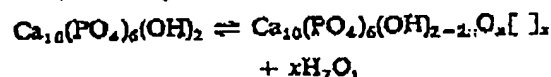
In spite of the differences among the various PS coatings, similarities can also be noticed. All the studied coatings were well crystallized. Both HAM and HAF PS coatings underwent dehydroxylation and decomposition to a mixture of apatite with α -TCP and tetra CP. Both β -TCPPM and β -TCPPD coatings transformed to α -TCP subsequent to PS. Thus the differences are primarily related to the degree of transformation.

The above plasma spraying induced transformations of either HA or β -TCP can be expected, because plasma spraying takes the particles through high temperatures. At temperatures above 1050 °C there exists equilibrium for HA with TCP and tetra CP as follows [16]:



The β -modification of TCP is stable at temperatures up to 1120 °C; in the range from 1120 °C and up to 1470 °C the α -modification of TCP is stable [7]. The transformation temperatures depend on partial water pressure, impurities and stoichiometry. In case of CPC applied as coatings on metal surface other factors may be involved shifting the above reaction toward destabilization of HA or lowering the β -TCP to α -TCP transition temperature. For instance, this might be a phenomenon similar to the effect of a titanium substrate upon sintering β -TCP: it was found that it promoted β - to α -TCP transformation [9].

There are several techniques to prevent the decomposition of HA or β -TCP during plasma spraying, such as using large starting particles, varying the PS atmosphere, and controlling plasma spraying parameters which minimize time of flight and temperature. This may lead to retaining a 95% apatitic structure as in the case of HAF PS. However, dehydroxylation of HA can hardly be prevented at the high plasma spraying temperatures. There is a likely dehydroxylation, according to the reaction:



where [] is a vacancy.

XRD analysis does

not reveal the dehydroxylation. The XRD patterns of the main components of both HA and PS are apatitic structure patterns (Fig. 3a, b). If the structural characterizations were only based on XRD analysis, one could conclude to a hydroxyapatite structure. Yet, the recorded IR spectra of the HA PS do not correspond to that of HA, but resemble that of OHA which was first described by Trombe and Montel [11]. They indicated that it is hardly possible to obtain stoichiometric oxyapatite stable in air at room temperature. One obtains solid solutions of HA and oxyapatite, with compositions closer to oxyapatite. A composition of a slightly hydroxylated OHA, i.e. one which does not produce the 3572 cm^{-1} OH^- band in the IR spectrum, corresponds to the formula



Upon hydroxylation, i.e. the increase of water content in the lattice of OHA, the IR spectrum gradually changes toward the one of HA. Specifically, a gradual increase of intensity of the 3572 and 633 cm^{-1} OH^- bands occurs with increasing hydroxylation [11].

The information contained in the characteristic spectral features of the OH^- group leads to the identification of important structural characteristics which cannot be made by XRD. In addition, the FTIR spectra also bring out features in the PO_4 bands energy region with structural information impossible to obtain by XRD. Two weak bands at 970 and 946 cm^{-1} replace the symmetric stretching band of the PO_4 group of HA at 961 cm^{-1} . Small extra bands for a bending mode of vibration (in the range from 604 to 556 cm^{-1}) and a medium intensity band at 480 cm^{-1} appear (Fig. 4a, b). In general, the number of bands attributable to PO_4 groups in apatites containing bivalent ions (O^{2-} , CO_3^{2-} , S^{2-}), is greater than the theoretical number for a crystal with hexagonal symmetry and spatial group $\text{P6}_3/\text{m}$. In the case of OHA (or other apatites with bivalent ions), a bivalent ion (O^{2-}) and a vacancy-substitute for two monovalent ions. The substitution leads to a distortion of the symmetry. Since XRD shows a hexagonal symmetry there must be statistical disorder of the bivalent ions and vacancies along the c -axis. The distortion, however, affects the infrared spectrum: even though the phosphate groups occupy identical positions in the HA or OHA lattice, there is a difference in terms of location with regard to the bivalent ions or vacancies in the lattice of OHA [11].

Plasma spraying also affects coating morphology and microporosity. We found that plasma spraying leads to a reduction in specific surface area for all of the studied coating apparently due to a combined effect of heat and impact. Plasma spraying changes the morphology of particles. Specifically, the HAF PS coating at high magnification appears to be a combination of large highly deformed particles with a glassy appearance, ceramic chips, randomly distributed pores, and cracks. These cracks could lead to local stress concentration and a crevice-like conditions which would result in mechanical and physico-chemical instability of the coating.

affected by plasma spraying. The Ca/P ratio of the coatings, estimated by measuring the Ca/P Auger peak intensity ratio on the surface of HAF PS, was found to correspond to that of stoichiometric HA used as a reference. Thus plasma spraying does not seem to change stoichiometry of the outer layer of the coatings. It is this layer which determines the initial solid-solution interfacial reactions and biological response upon implantation.

4.2. *In vitro* stability

Controversial reports on the degree of plasma sprayed coating resorption [14, 18–20] suggest dependency on the process parameters. In the present study we determined the dissolution rates of the plasma sprayed coatings in calcium and phosphate free buffer solution at physiological pH. The dissolution rates of all the studied ceramic coatings were found to be greater than those of the starting particles. Both, the initial dissolution rates, as well as the Ca and P concentrations in solution appeared to increase. The observed increase in the dissolution rates occurs in spite of the plasma sprayed induced decrease in specific surface areas.

The dissolution rates of both HAM PS and HAF PS exceeded that of starting β -TCP which is known as a soluble, metastable and resorbable member of the CPC family. Even HAF PS, which is actually 95% apatite, was found to be more soluble than the starting β -TCP. The dissolution rates of the HAM PS and β -TCPM PS were found to be significantly greater than the starting HAM and β -TCP (Fig. 7a), and both showed the greatest solubility among the studied compounds, either starting or plasma sprayed. The dissolution rates of both studied β -TCP PS were found significantly enhanced compared with that of the starting powders.

Among the main factors which have been forwarded to explain solubility of CPC at constant pH are the Ca/P ratio [16], porosity and microporosity [21] (specific surface area). Since these factors are either unchanged, or work oppositely, the main factor to consider is the plasma spraying induced phase transformations. Specifically, a greater solubility of HAF PS compared with the starting HAF is apparently related to the transformation of HA to OHA. Furthermore, important increases in solubility occur when single phase HA or β -TCP decompose into α -TCP containing mixtures, $\text{OHA} + \alpha\text{-TCP} + \text{tetra CP}$, or $\alpha\text{-TCP} + \beta\text{-TCP}$ respectively. The dissolution experiment with single-phase OHA and α -TCP made by us proves that the transformations of HA into OHA and β -TCP into α -TCP lead in fact to significant increases in the dissolution rates (Fig. 8a, b).

The solubility of OHA was expected to be high on the basis of thermodynamic considerations [22]. The current data show that the Ca release rate from OHA in a Ca-free buffer solution at pH 7.3 is comparable with that of β -TCP (Fig. 8a). The higher solubility of OHA is probably related to the distortion of the bivalent ions

(O^{2-}) and vacancies. A crystal structure disorder following from the high temperature rearrangements occurring during β - to α -TCP transformation is apparently responsible for the greater solubility of α -TCP.

Significant rates of resorption and degradation of plasma sprayed CPC coatings reported in a number of studies [20, 23, 24] can be related to the herewith reported spraying induced enhancement of *in vitro* dissolution rates. Specifically, the observed $10 \mu\text{m wk}^{-1}$ resorption rate of plasma sprayed β -TCP coating [23] may be explained by transformation of the original structure to α -TCP, as reported by the authors. In a later report [14] a group of authors from the same laboratory argued that the resorption rate of β -TCP PS was not as great as was reported before [23]; however, they did not mention the actual structure of the coating in this more recent study.

The resorption rate of a plasma sprayed HA coating consisting of "over 98% highly crystalline HA as confirmed by XRD" was found to be $50 \mu\text{m}$ in few first months [20]. However, since dense HA is generally described as "non-resorbable", we can assume that the reported significant resorption of the coating with 98% HA was probably related to dehydroxylation and transformation of insoluble HA to metastable OHA during plasma spraying. The 2% remaining unidentified structural components, which were apparently α -TCP and tetra CP can also lead to an additional enhancement of resorbability.

Plasma sprayed HA and β -TCP showed no significant difference in degradation rates in Ringer's and saline [24]. Although the authors did not report on the resultant coating structures, decomposition of HA with a formation of α -TCP and transformation of β - to α -TCP which are likely to occur during plasma spraying may have lead to an almost similar instability of the HA and β -TCP PS coatings [24].

5. Conclusions

All the commercially obtained coatings underwent significant plasma sprayed induced changes in crystal structure, phase composition, specific surface area and morphology. The phase transformations depended on the starting particle characteristics. Specifically, the starting β -TCP transformed to variable concentrations of α -TCP. The starting HA was dehydroxylated and transformed to OHA, and partly decomposed to α -TCP and tetra CP.

HA and OHA can not easily be differentiated by using XRD, however, they can easily be identified as different compounds by using infrared analysis.

The plasma sprayed phase transformations produce significant increases in *in vitro* dissolution rate of both plasma sprayed HA and TCP coatings. All HA coatings including the coating with a 95% highly crystalline apatitic structure showed significantly enhanced dissolution rates.

Specimens were kindly provided by the companies listed in Table I.

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